

Monitoring Genotoxic Exposure in Uranium Mines

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Recent data from deep uranium mines in Czechoslovakia indicated that miners are exposed to other mutagenic factors in addition to radon daughter products. Mycotoxins were identified as a possible source of mutagens in these mines. Mycotoxins were examined in 38 samples from mines and in throat swabs taken from 116 miners and 78 controls. The following mycotoxins were identified from mines samples: aflatoxins B₁ and G₁, citrinin, citreoviridin, mycophenolic acid, and sterigmatocystin. Some mold strains isolated from mines and throat swabs were investigated for mutagenic activity by the SOS chromotest and Salmonella assay with strains TA100 and TA98. Mutagenicity was observed, especially with metabolic activation *in vitro*. These data suggest that mycotoxins produced by molds in uranium mines are a new genotoxic factor for uranium miners.

Introduction

In 1990 various methods of biomonitoring were used to examine genotoxicity in groups of miners from uranium mines. Significantly increased frequencies of chromosome aberrations in peripheral lymphocytes, decreased unscheduled DNA synthesis (UDS) in lymphocytes, as well as increased lipid peroxidation (LPO) in plasma and lymphocytes were found in uranium miners in comparison to controls (1). It was not possible to explain observed changes only as the effect of radon daughter products (2). The spectrum of chromosome aberrations corresponded to the effect of chemical mutagens.

In the same mines, the mutagenicity of runnel water as well as drilling water were observed using Ames indicator strain TA98 with and without metabolic activation (3). An unexpected risk factor may be toxic metabolites of molds to the miners. Warm temperature and high humidity are factors stimulating the growth of these molds. The genotoxicity of various mycotoxins and metabolites may be another risk factor responsible for the continued high cancer risk of these miners.

In 1970, Kusak et al. (4,5) indicated the influence of biological factors in the pathogenesis of Joachimstal disease in miners from uranium mines—a possible role of *Aspergillus flavus*, which produces carcinogenic aflatoxin. In 1989 Dobias et al. (6) observed the mutagenic activity of metabolites produced by *Aspergillus* and *Penicillium* in ore mines with increased radon exposure.

A common denominator of uranium and ore mines is increased radioactivity. Ionizing radiation affects not only humans, it may similarly act as a selective factor for populations of various molds. This selective factor—affecting morphology, growth, and metabolism of industrial microorganisms—is well known. However, information about the effect of ionizing radiation on mold metabolism in the environment is scarce. Adamek (unpublished observations, 1970) postulated that molds absorb radioactive substances and pass them on to the conidia. Laryngeal smears from the respiratory tracts of uranium miners contained not only *Aspergillus flavus* but also a series of its mutants (5). Experimental data indicate that long-term exposure to dust, contaminated by toxic products of molds, increases the risk of cancer in respiratory system. The size of conidia is optimal for its deposition in lungs (7). We hypothesized that mutagenic mycotoxins may be another risk factor responsible for genotoxicity observed in uranium miners. We report here the results of toxicity and mutagenicity of throat swab samples from uranium miners.

To reduce exposure to radon daughter products, ventilation in these mines was increased substantially in the period 1968–1972. This increase of ventilation could also increase the spread of mold conidia in uranium mines, but such a possibility has not been studied.

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Materials and Methods

Samples from Mines

First samples were taken in February 1990 at the ninth-floor at mine Vitkov II-Tachov. Ten swabbed samples were obtained.

Another set of samples was taken in February and March 1991 at the 25th floor of mine no. 19 at Příbram. Samples were obtained by swabbing from walls and woods, using sterile wires from anticorrosive steel with cotton. Before swabbing, they were touched in sterile physiological solution. If mold grew on wood, the contaminated part was cut down. Twenty-five samples were collected.

Throat Swab Samples

Throat swabs samples were obtained in June 1991 in Příbram. Subjects were 116 miners, mean age 33.8 ± 4.8 years. The control group consisted of firemen, policemen, and iron workers, mean age 36.1 ± 9.8 years. Throat swab samples were taken using sterile wires from anticorrosive steel with cotton. Before swabbing, they were touched in a sterile physiological solution.

Mycotoxin Identification

All samples from mines and all throat swab samples were cultivated on Czapek-Dox agar, Sabaraud's agar with 7% NaCl. Colonies of molds were estimated according to genus *Aspergillus*, *Penicillium*, and *Fusarium*. The mold isolates were cultivated for 14 days at 25°C on 10% saccharose and 1% yeast autolysate and subsequently extracted into chloroform. One part of extract was used to determine the embryotoxicity on 40-hr-old chick embryos (8). The other part was used for thin-layer chromatographic determination of 23 mycotoxins (8): aflatoxins B₁ and G₁, brevianamid A, citrinin, citreoviridin, cytochalasin E, cyclopiazonic acid, deoxynivalenol, diacetoxyscirpenol, fusaric acid, griseofulvin, luteoskyrin, mycophenolic acid, ochratoxin A, patulin, penicilic acid, PR-toxin, rubratoxin B, rugulosin, sterigmatocystin, secalonin acid, T-2 toxin and zearalenon.

Mutagenicity and Mold Extracts

The mold isolates were cultivated for 5 days at 25°C on 10% saccharose and 1% yeast autolysate and subsequently extracted into chloroform. Samples were evaporated and resuspended in 4 mL of dimethylsulfoxide for mutagenicity testing.

Ames Test

The mutagenic effects of the extract from the molds were analyzed using *Salmonella typhimurium* strains TA100 and TA98 (9,10). The indicator strains were obtained from B. N. Ames. The genetic properties of all bacterial cultures were checked under conditions recommended by Maron and Ames (10).

The S-9 and S-9 mix for the tests with metabolic activation were prepared following the procedure of Ames et al. (9). The S-9 from male Wistar rats pretreated by Delor 103 (compound analogous to Aroclor 1254) contained 30 mg of protein/mL [determined according to Lowry et al. (11)].

Standard plate incorporation assay with *Salmonella typhimurium* strains (with and without S-9) were performed as described by Maron and Ames (10). The extracts of the molds were dissolved in 100% dimethylsulfoxide (SERVA, FRG) and were added to agar plates at 100, 50, and 20 μ L per plate. Plates were incubated for 48 hr at 37°C.

SOS Chromotest

The extracts from molds were tested by a quantitative bacterial colorimetric assay. The indicator strain was obtained from P. Quillardet. The *E. coli* PQ37 tester strain used in the SOS chromotest carries a *sfia::lacZ* fusion with a deletion for the normal *lac* region. Therefore, β -galactosidase activity is strictly dependent on *sfia* expression (12).

The standard procedure was performed as described by Quillardet and Hoffnung (12). S-9 was of the same origin as in the Ames test. The extracts of molds were dissolved in dimethylsulfoxide, they were added in volumes from 5 to 50 μ L per test tube.

Results

The results of SOS chromotest and *Salmonella* assay from mold extracts of the Vitkov mine are shown in Table 1. From 10 samples were isolated 13 various molds, 8 of which carried mutagenic products, especially after metabolic activation.

The results of mycotoxin analysis from mine at Příbram are shown in Table 2. From 25 samples were isolated 90 different molds, 65 of which were toxic. From these molds, 12 strains (8 with a high level of the mycotoxin production,

Table 1. Mutagenic activity of molds in bacterial short-term tests (uranium mine Vitkov II-Tachov).

Sample	Mold	Mutagenic activity					
		SOS chromotest		Salmonella assay			
		-S9	+S9	TA98		TA100	
1A	<i>Aspergillus</i>	+	+	-	+	+	+
1B	<i>Penicillium</i>	-	-	-	-	-	-
2	<i>Streptomyces</i>	-	-	-	-	-	-
3	<i>Aspergillus</i>	+	+	+	+	+	+
4A	<i>Penicillium</i>	+	+	+	+	-	+
4B	<i>Aspergillus</i>	+	+	-	+	-	+
5	<i>Aspergillus</i>	-	+	+	+	+	+
6	<i>Aspergillus</i>	-	+	-	+	-	-
7	<i>Aspergillus</i>	+	+	+	+	+	+
8	<i>Aureobasidium</i>	-	-	-	-	-	-
9	<i>Streptomyces</i>	-	-	-	-	-	-
10A	<i>Aspergillus</i>	-	+	-	+	-	+
10B	<i>Penicillium</i>	-	-	-	-	-	-
Standard aflatoxin		-	+	-	+	-	+
Negative control		-	-	-	-	-	-

Table 2. Toxinogenic activity of molds from uranium mine (Pribram).

Type of mold	<i>n</i>	Toxinogenicity ^a			
Penicillium	37	11	+	+	+
		22		+	+
		4		0	
Aspergillus	24	14	+	+	+
		7		+	+
		3		0	
Fusarium	9	5	+	+	+
		3		+	+
		1		0	
Others	20	1	+	+	+
		2		+	+
		17		0	
Samples	25				
Samples with molds	25				
Isolated molds	90				
Toxinogenic molds	65 (72%)				

^a(+ + +) High level of mycotoxin production; (+ +) mid-level of mycotoxin production; 0, no mycotoxin production.

4 with a low one) were randomly chosen for analysis of mutagenic activity of their metabolites in Ames test. The mutagenicity was observed in five strains, three of them with a high and two with a low level of mycotoxin production (Table 3).

Similarly 15 strains were chosen from throat smears of uranium miners: 5 samples with a high level of mycotoxin production, 5 with a low one, and 5 without any toxic activities (Table 4). Mutagenicity was observed in 4 samples from strains with high as well as low level mycotoxin production.

Discussion

Our study revealed an unexpected factor in uranium mines—the increased occurrence of molds (genus *Aspergillus*, *Penicillium*, and *Fusarium*) that produce mycotoxins. A possible genotoxic hazard for uranium

Table 3. Mutagenic activity of molds from uranium mines.

Mold	Toxinogenicity ^a	Mycotoxin	Salmonella assay			
			TA 100		TA 98	
			– S9	+ S9	– S9	+ S9
<i>Penicillium cyclopium</i>	+	MA	–	–	–	–
<i>Aspergillus flavus</i>	+	AFB ₁ , G ₁	–	–	–	+
<i>A. tamari</i>	+	?	–	–	–	–
<i>Fusarium solani</i>	+	?	–	+	–	+
<i>P. expansum</i>	+	?	–	–	–	–
<i>P. citrinum</i>	+	Citrinin	–	–	–	–
<i>P. citreo-viridae</i>	+	Citreoviridin	–	–	–	–
<i>A. versicolor</i>	+	Sterigmatocystin	+	+	–	+
<i>A. ustus</i>	+	?	–	–	+	–
<i>A. ustus</i>	+	?	–	+	–	–
<i>Paecilomyces</i> sp.	+	?	–	–	–	–
<i>Stachybotris</i> sp.	+	?	–	–	–	–

Abbreviations: MA, mycophenolic acid; AFB₁, G₁, aflatoxin B₁, G₁; ?, unidentified mycotoxins.

^a(+ + +) high level of mycotoxin production; (+) low level of mycotoxin production.

Table 4. Mutagenic activity of mold strains from throat smears of uranium miners.

Mold	Toxinogenicity ^a	Mycotoxin	Salmonella assay			
			TA 100		TA 98	
			– S9	+ S9	– S9	+ S9
<i>Penicillium rugulosum</i>	+	Rugulosin	–	–	–	–
<i>P. fellutanum</i>	+	Sterigmatocystin	–	–	–	–
<i>Aspergillus versicolor</i>	+	Sterigmatocystin	–	+	–	+
<i>P. cyclopium</i>	+	PA	–	–	+	–
<i>P. expansum</i>	+	PA	–	–	–	–
<i>P. candidus</i>	+	?	–	–	–	–
<i>A. ustus</i>	+	?	–	–	–	–
<i>A. geaucus</i>	+	?	–	–	–	–
<i>A. restrictus</i>	+	?	–	+	–	–
<i>P. frequentans</i>	+	?	–	–	+	–
<i>Trichoderma</i> sp.	–	–	–	–	–	–
<i>Acremonium</i> sp.	–	–	–	–	–	–
<i>Botrytis cinerea</i>	–	–	–	–	–	–
<i>Cladosporium</i> sp.	–	–	–	–	–	–
<i>Doratomyces</i> sp.	–	–	–	–	–	–

Abbreviations: PA, penicilic acid; ?, unidentified mycotoxin.

^a(+ + +) high level of mycotoxin production; (+) low level of mycotoxin production.

miners may be expected, as mutagenic activity of these mold metabolites was observed in Ames test.

High mutagenic activities of produced metabolites (e.g., from a strain of *Fusarium solani*) was detected in *Salmonella* tester strains. The mutagenic activity in strain TA100 was three to six times and in strain TA98 three to seven times more revertants than in control. In both cases, mutagenicity was only demonstrated with metabolic activation *in vitro*.

The mutagenicity of throat swab samples was observed only in four cases. According to literature data, one should not expect to detect mutagenicity in all toxic strains (13,14). There is a difference in produced mycotoxins, and mutagenic activity may be lost due to cultivation of strains on synthetic media. Results given in Table 4 indicate that microflora from throats of miners produced mycotoxins with a high genotoxic potential. This phenomenon was not observed in the control group.

Several years ago, an increased risk of laryngeal cancer was detected at the supraglottic location among young uranium miners (15). We propose that mycotoxins identified in mines and in miners' throats and the mutagenic activity of these mycotoxins support the clinical observation. Most significant is the finding of aflatoxin B₁, sterigmatocystin, rugulosin, citrinin, and penicillic acid, which are mutagenic and have some evidence of carcinogenic activity (16,17).

REFERENCES

1. Šrám, R. J., Bínková, B., Dobíáš, L., Rössner, P., Topinka, J., Veselá, D., Veselý, V., Stejskalová, J., Bavorová, H., and Řeřicha, V. Monitoring genotoxic exposure in uranium miners. *Environ. Health. Perspect.* 99: 303-305 (1993).
2. Sevc, J., Kunz, E., Tomasek, L., Placek, V., and Horacek, J. Cancer in man after exposure to Rn daughters. *Health Phys.* 54: 27-46 (1988).
3. Cerna, M., Pastorkova, A., Rossner, P., Dobias, L., and Sram, R. J. Mutagenic activity analysis of factors in the working environment of uranium mines [In Czech]. *Priloha AHEM* 13: 101-104 (1990).
4. Kusak, V. Ethioopathogenesis of bronchogenic pulmonary carcinoma in miners from uranium mines. *Strahlentherapie* 138: 549-555 (1969).
5. Kusak, V., Jelinek, S., and Sula, J. Possible role of *Aspergillus flavus* in the pathogenesis of Schneeberg and Jachymov disease. *Neoplasma* 17: 441-449 (1970).
6. Dobias, L., Gajdosova, D., Sram, R. J., Janca, L., and Roda, S. Risk associated with inhalation of fungal metabolites (abstract). Presented at the International Conference on Genetic Toxicology of Complex Mixtures, July 4-7, 1989. Fifth ICEM Satellite Meeting, Washington, DC, 1989, p. 37.
7. Zennie, T. M. Identification of aflatoxin B₁ in grain elevator dusts in central Illinois. *J. Toxicol. Environ. Health* 13: 589-593 (1984).
8. Vesely, D., Vesela, D., and Jelinek, R. Use chick embryo in screening for toxin-producing fungi. *Mycopathologia* 88: 135-140 (1984).
9. Maron, D. M., and Ames, B. N. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* 113: 173-215 (1983).
10. Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.* 31: 347-364 (1975).
11. Lowry, D. H., Rosebrough, N. J., Fan, A. L., and Randall, R. J. Protein measurement with folin phenol reagents. *J. Biol. Chem.* 193: 265-275 (1951).
12. Quillardet, P., and Hoffnung, M. The SOS Chromotest, a colorimetric bacterial assay for genotoxin producers. *Mutat. Res.* 147: 65-78 (1985).
13. Auffray, Y., and Boutibonnes, P. Evaluation of the genotoxic activity of some mycotoxins using *Escherichia coli* in the SOS spot test. *Mutat. Res.* 171: 79-82 (1986).
14. Stark, A. A. Mutagenicity and the carcinogenicity of mycotoxins: DNA binding as a possible mode of action. *Ann. Rev. Microbiol.* 34: 235-263 (1980).
15. Tichý, S., and Janisch, R. The incidence of laryngeal carcinoma in uranium mine workers [in Czech]. *Pracov. Lék.* 25: 428-430 (1973).
16. Bautybanes, P., Auffray, Y., Malherbe, C., Kogbo, W., and Marais, C. Propriétés antibacteriennes et genotoxines de 33 mycotoxines. *Mycopathologia* 87: 43-49 (1984).
17. IARC. Monographs on the Evaluation of Carcinogenic Risk to Humans: An Updating of IARC Monographs Vols. 1-42, Supplement 7. International Agency for Research on Cancer, Lyon, 1987.